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Increased circulating regulatory T cells in medicated people with schizophrenia

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ABSTRACT

Immunological abnormalities are increasingly reported in people with schizophrenia, but no clear functional biomarkers associated with genetic correlates of the disease have been found. Regulatory T cells (Tregs) are key immunoregulatory cells involved in the control of inflammatory processes and their functions are directly related to the human leucocyte antigen (HLA) gene, which has been implicated in schizophrenia genetic studies. However, there is a lack of studies reporting Treg status in people with schizophrenia. In the current study, the proportion of circulating Tregs was examined using flow cytometry in 26 medicated participants with schizophrenia and 17 healthy controls. Psychiatric symptoms and cognitive function were evaluated using the Scale for the Assessment of Negative Symptoms, the Brief Psychiatric Rating Scale, and the MATRICS Consensus Cognitive Battery. The proportion of Tregs was found to be significantly greater in the schizophrenia group compared to healthy controls. No differences were observed in total lymphocyte counts or $CD3^+$ and $CD4^+$ T cells, confirming a specific effect for Tregs. Elevated Tregs in schizophrenia correlated with fewer negative symptoms, a core domain of the illness. These results suggest that Tregs may contribute to improved negative symptoms in schizophrenia, possibly by counteracting on-going inflammatory processes.

1. Introduction

Schizophrenia (SZ) is a complex heterogeneous disease with recognized genetic risk factors. Genetic studies have identified more than 100 loci conferring risk for the disease and there is consensus today for a polygenic etiology of SZ (Purcell et al., 2009). Genome wide association studies (GWAS) using large cohorts have strongly implicated polymorphisms in the chromosome 6p21.3–22.1 region as one the most consistent genomic regions associated with the disease (Agartz et al., 2011; Bergen et al., 2012; Stefansson et al., 2009; Zhang et al., 2013). This region corresponds to a highly variable and recombinant portion of the human genome coding for human leucocyte antigen (HLA) proteins, which are also known as major histocompatibility complex (MHC) molecules (Stefansson et al., 2009). Due to their involvement in cellular immune functions, including antigen processing and tolerance, it is believed that the pathophysiology of SZ is related, at least in part, to disrupted immune processes that influence the responses of the immune system and its interaction with the central nervous system, with particular effects during prenatal neurodevelopment (Brown, 2011; Elmer and McAllister, 2012; McAllister, 2014). Indeed, there are an increasing number of studies pointing to an ongoing inflammatory process in SZ characterized by elevated inflammatory markers in peripheral blood (Doorduin et al., 2009; Fineberg and Ellman, 2013; Muller et al., 2012; Saetre et al., 2007).

Cytokines, chemokines and C-reactive protein are among the most commonly studied immune function biomarkers in SZ (Brown et al., 2004; Fatjo-Vilas et al., 2012; Fineberg and Ellman, 2013; Hope et al., 2013; Miller et al., 2013a; Reale et al., 2011). These molecules have gained relevance in clinical research as they may contribute to the underlying pathophysiological processes and genetic underpinnings associated with SZ. Nevertheless, cytokines and chemokines are involved in multiple cellular functions that may or may not be directly linked with MHC function. Thus, immune biomarkers directly related with MHC function may provide a more stable biomarker, as well as provide potential mechanistic insights on specific pathological cellular processes.

In this regard, $CD3^+$ T cells are directly related to MHC function; these cells depend on permanent interactions involving T cell receptor-

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MHC complexes during development, differentiation and survival of the T cell repertoire (Charles et al., 2001). Thus, $CD3^+$ T cells may constitute a valuable biomarker of the disease in relation to functional variation of the MHC locus. Moreover, increasing evidence from basic rodent studies implicates different types of $CD3^+$ T cells in particular brain functions and behavioral processes (Kipnis et al., 2008; Kipnis et al., 2012), which may provide specific insights about endophenotypes or symptoms of the disease. However, studies reporting on various aspects of $CD3^+$ T cell biology in SZ are relatively uncommon (Craddock et al., 2007; Ding et al., 2014; Drexhage et al., 2011; Fernandez-Egea et al., 2016; Miller et al., 2013b). $CD3^+$ T cells differentiate into two major types, expressing one of two different cell surface markers, CD8 or CD4, which allow specific interactions with either MHC class I or class II respectively. Within the $CD4^+$ type, regulatory T cells ($CD4^+/CD25^+/Foxp3^+$; Tregs) are an attractive $CD3^+$ T cell subtype for a biomarker in SZ, since they function to provide important immunomodulatory control over other T cell subtypes and inflammatory processes (Arce-Sillas et al., 2016; Miyara et al., 2009; Rodriguez-Perea et al., 2016). The development and functions of these cells are directly related to the expression of the transcription factor Foxp3 and the CD25 cell surface marker within the $CD3^+/CD4^+$ T cell subtype (Kitagawa and Sakaguchi, 2017; Mohr et al., 2018). Tregs have broad immune suppressive activity on diverse immune cells, which requires MHC cellular interactions. Therefore, in the present study we compared the proportions of Tregs in healthy controls and stable medicated participants with SZ and examined the relationship between Tregs and negative symptoms and cognitive performance, two core domains of the illness.

2. Methods

2.1. Study participants and data and sample collection

All participants were recruited from the Maryland Psychiatric Research Center, University of Maryland in Catonsville, MD between February and November of 2017. Participants were between 18 and 64 years of age and of either sex and any race. Participants with schizophrenia were required to meet Diagnostic and Statistical Manual (DSM)-5 criteria (American Psychiatric Association, 2013) for schizophrenia or schizoaffective disorder, able to give informed consent and considered clinically stable as defined by use of the same antipsychotic treatment for 30 days prior to the study. Healthy controls were included if they were free of all major psychiatric illness as determined by the Structured Clinical Interview for Diagnosis-5, and able to give informed consent. Exclusion criteria for both participants with schizophrenia and healthy controls included a DSM-5 diagnosis of alcohol or substance misuse disorders currently or in the last 3 months, any current active systemic infection with fever (temperature > 38 °C), any long-term untreated chronic infections, pregnancy, and other conditions whose pathology and treatments may alter their immune status. The study was approved by the University of Maryland Institutional Review Board (IRB), reliance was granted to UMB IRB by the Department of Health and Mental Hygiene IRB, and the study was approved by the Spring Grove Hospital Research Committee. Twenty-six participants with schizophrenia and 17 healthy controls met inclusion and exclusion criteria, passed the Evaluation to Sign Consent (ESC), signed informed consent and were enrolled in the study.

2.2. Laboratory assays

All study participants arrived between 7:00 and 8:00 am under fasting conditions. Participants had their blood drawn for flow cytometry, a complete blood count (CBC) and a Chemistry 14 panel. All processing of blood was completed immediately. Samples were maintained at 2–5 °C and processed for flow cytometry within 2 h of collection using a stringent collection and transport protocol. Samples for the CBC and Chemistry 14 were sent to LabCorp (Burlington, NC, USA) for standard processing. In addition to the blood draws, demographic information, height, weight, sex/gender, past medical history, current medications, and blood pressure data were collected on the same day.

2.3. Neuropsychological and psychiatric symptom evaluation

The MATRICS Consensus Cognitive Battery (MCCB) was used to assess neuropsychological test performance. The MCCB is specifically designed to assess cognition in people with schizophrenia. The MCCB is comprised of 10 tests, which assess seven cognitive domains. The MCCB composite score is a standardized mean of the seven domain scores. T-scores are standardized to normative data, and have an estimated mean of 50 and SD of 10 in the general healthy population (Nuechterlein et al., 2008).

The Brief Psychiatric Rating Scale (BPRS) total score was used to measure global psychopathology. The four BPRS positive symptom items - conceptual disorganization, suspiciousness, hallucinatory behavior, and unusual thought content – were used to measure positive psychotic symptoms (Overall, 1962). The Scale for the Assessment of Negative Symptoms (SANS) minus the global items, inappropriate affect, poverty of content of speech, and attention items, was used to evaluate negative symptoms (Andreasen, 1982; Buchanan et al., 2007). The Calgary Depression Rating Scale (CDS), which was specifically designed to assess depressive symptoms in people with schizophrenia (Addington et al., 1993) was used to measure depressive symptoms. Finally, the Clinical Global Impression (CGI) severity item was used to assess global illness severity (Guy, 1976). The MCCB and symptom assessments were conducted immediately after the blood samples were obtained.

2.4. Flow cytometry and T cell analyses

Peripheral blood mononuclear cells (PBMCs) were prepared using Ficoll density gradient separation following manufacture's guidelines (Ficoll-Paque™ PLUS Media, GE Healthcare™). Briefly, after centrifugation the mononuclear cell layer was washed twice and re-suspended in 1 ml MACS buffer (1X PBS, 2% FBS, 2 mM EDTA). After determining the cell concentration, approximately $1–1.5 \times 10^6$ cells were washed twice with PBS and re-suspended in 1 ml PBS buffer and 1 µl viability marker (Fixable Viability Dye eFluor™ 506, eBioscience, Cat#: 65-0866-14). Cells were then incubated at 4 °C in the dark for 30 min and washed twice with PBS. Cell surface epitopes for CD3, CD4, CD25, and CD45RA were labeled before fixation. For this, cells were re-suspended in 100 µl PBS buffer containing a combination of fluorescence conjugated anti-human antibodies including: PE-CD3 (BioLegend Catalog #: 317,308); FITC−CD4 (BioLegend Catalog #: 357,405); PE/Cy7-CD45RA (BioLegend Catalog #: 304,126); PerCP-Cy5.5; and CD25-APC (Miltenyi Biotec Inc. Catalog #: 130-098-213). Cells were incubated at 4 °C in the dark for 30–60 min. After surface staining, the cells were fixed and permeabilized overnight at 4 °C, washed again and then labeled for Foxp3 with e-fluor 450-Foxp3 (FOXP3 Monoclonal Antibody (236A/E7), eBioscience™, Catalog #: 48-4777-42) at 4 °C in the dark for 1 h. The samples were then washed and resuspended in fresh buffer for analysis. Flow cytometric analysis was performed using a BDTM LSR II flow cytometer (BD Biosciences, San Jose, California). Lymphocytes were gated on forward scatter (FSC) versus side scatter (SSC), corrected for live/dead and single cells (Fig. 1A–C), and 500,000 events collected for initial gating and then standardized to 50,000 lymphocytes for analysis using FlowJo version 10 software (Tree Star, Ashland, Oregon) with biexponential scaling. Tregs were quantified within the $CD3^+$ and $CD4^+$ gate as $CD25^+/Foxp3^+$ (Fig 1A, B). Tregs negative for the CD45RA antigen were defined as activated Tregs (Fig. 1C) and used as marker of functional Tregs (Miyara et al., 2009). Blood from two collection tubes per participant were processed individually. This generated duplicate data per single participant.